Quick-Start Protocol April 2016

PyroMark® Q24 Advanced Reagents – Part 2

See *Quick-Start Protocol: PyroMark Q24 Advanced Reagents – Part 1* for instructions about kit storage and additional necessary equipment and reagents.

Further information

- *PyroMark Q24 Advanced Reagents Handbook*: **www.qiagen.com/HB-1433**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- These protocols describe preparation of single-stranded template and annealing to primer, and starting the PyroMark Q24 Advanced run. See *Quick-Start Protocol: PyroMark Q24 Advanced Reagents – Part 1* for immobilization of biotinylated PCR product to beads and loading the PyroMark Q24 Cartridge with reagents.
- Use a room temperature (15–25°C) PyroMark Q24 Plate Holder when preparing and moving the plate.
- Ensure that the PyroMark Q24 Vacuum Workstation is prepared for sample preparation, as described in the *PyroMark Q24 Advanced User Manual*.
- Perform the function test for the filter probes as described in the *PyroMark Q24 Advanced User Manual* regularly and exchange filter probes when indicated.

Protocol 3: Preparation of template DNA and annealing to primer

1. Dilute a sufficient amount of each sequencing primer to 0.375 µM in PyroMark Advanced Annealing Buffer.
2. Add 20 µl of diluted sequencing primer to each well of the PyroMark Q24 Plate according to the run setup.

3. Place the PCR plate from Protocol 2 and the PyroMark Q24 Plate on the vacuum workstation (Figure 1). Ensure that the plate is in the same orientation as when the samples were loaded. Inspect the PCR plate and ensure the Sepharose beads are in solution.

Figure 1. Placement of PCR plate and PyroMark Q24 plate on the vacuum workstation.

4. Switch on the vacuum pump of the PyroMark Q24 Vacuum Workstation. Apply vacuum to the tool by switching on the vacuum.
5. Slowly lower the filter probes of the vacuum tool into the PCR plate to capture the beads containing immobilized template. Hold the probes in place for 15 s. Take care when picking up the vacuum tool.

   Note: Sepharose® beads sediment quickly. Capturing of beads must take place immediately following agitation. If more than 1 min has elapsed since the plate was agitated, agitate again for 1 min before capturing the beads.
6. Inspect the plate for a complete take up of all samples by the vacuum tool.
7. Transfer the vacuum tool to the trough containing 40 ml 70% ethanol (Figure 1, trough 1). Flush the filter probes for 5 s.
8. Transfer the vacuum tool to the trough containing 40 ml Denaturation Solution (Figure 1, trough 2). Flush the filter probes for 5 s.
9. Transfer the vacuum tool to the trough containing 50 ml Wash Buffer (Figure 1, trough 3). Flush the filter probes for 10 s.
10. Raise the vacuum tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes (Figure 2).

Figure 2. Illustration of the vacuum tool raised to beyond 90° vertical.

11. While holding the tool over the PyroMark Q24 Plate, close the vacuum switch on the tool (Off).
12. Release the beads into the PyroMark Q24 Plate by lowering the filter probes into the diluted sequencing primer and moving the tool gently from side to side.

    Note: Take care not to damage the surface of the PyroMark Q24 Plate by scratching it with the filter probes.
13. Transfer the vacuum tool to the trough containing high-purity water (Figure 1, trough 4) and agitate it for 10 s.
14. Wash the filter probes by lowering the probes into high-purity water (Figure 1, trough 5) and applying vacuum. Flush the probes with 70 ml high-purity water.
15. Raise the vacuum tool to beyond 90° vertical for 5 s, to drain liquid from the filter probes.
16. Close the vacuum switch on the tool (Off), and place the vacuum tool in the Parking (P) position.
17. Turn off the vacuum pump.

    Note: At the end of a working day, liquid waste and remaining solutions should be discarded and the PyroMark Q24 Vacuum Workstation should be checked for dust and spillage.
18. Heat the PyroMark Q24 Plate containing the samples at 80°C for 5 min using the pre-warmed PyroMark Q24 Plate Holder.
19. Remove the hot plate holder together with the PyroMark Q24 Plate from the heating block and place the plate immediately on the heating block of the PyroMark Q24 Advanced Instrument. Ensure that the plate-holding frame is closed.

    Note: The time between removing the plate holder from the heating block and placing the PyroMark Q24 plate in the instrument should not exceed 30 s.

20. Proceed directly with “Protocol 4: Running the PyroMark Q24 Advanced”.

Protocol 4: Running the PyroMark Q24 Advanced

1. Make sure that the PyroMark Q24 Cartridge was properly loaded and inserted into the PyroMark Q24 Advanced instrument (see protocol 2).
2. Make sure that the PyroMark Q24 Plate containing single-stranded template DNA with primer was properly placed on the heating block of the PyroMark Q24 Advanced instrument (see protocol 3). Close the instrument lid.
3. Insert the USB stick (containing the run file) into the USB port at the front of the instrument.

   Note: Do not remove the USB stick before the run is finished.
4. Select **Run** in the main menu (using the ▲ and ▼ screen buttons) and press **OK**.
5. Select the run file using the ▲ and ▼ screen buttons.

   To view the contents of a folder, select the folder and press **Select**. To go back to the previous view, press **Back**.
6. When the run file is selected, press **Select** to start the run.
7. When the run is finished and the instrument confirms that the run file has been saved to the USB stick, press **Close**. Remove the USB stick.
8. Open the instrument lid. Open the cartridge gate and remove the reagent cartridge by lifting it up and pulling it out. Close the gate.
9. Open the plate-holding frame and remove the plate from the heating block. Close the plate-holding frame and the instrument lid.
10. Discard the plate and clean the cartridge, according to the cleaning instructions in the product sheet supplied with the cartridge.
11. Analyze the run according to the *PyroMark Q24 Advanced User Manual*.

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